

Reproductive toxicity of pesticides on soil microarthropod fauna as ecotoxicological tool.

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ABSTRACT : Agricultural doses of heptachlor 20EC (16.25 lit/ha) and endosulfan 35EC (2.5 lit/ha) produced quick knockdown effect on 4 different nontarget soil microarthropod species under microcosm conditions. Short term direct toxicity decreased sharply with subagricultural dilutions but *Cyphoderus javanus* (Collembola) suffered considerable mortality even at 10,000 times dilution of the recommended agricultural dose of endosulfan 35EC. Both *C. javanus* and *Archegozetes longisetosus* (Cryptostigmata) were sensitive to heptachlor 20EC upto 300 times dilution of the agricultural dose. Sublethal toxicity of both pesticides on the reproductive behaviour was evident from reduced fecundity of all the 4 species investigated including highly sclerotised cryptostigmatid mites namely *Lancetoppia confusaria* and *Scheloribates albitalatus*. Chronic toxicity of endosulfan at 12,000 times dilution and heptachlor at 2,000 times dilution of the agricultural doses significantly reduced the fecundity of *C. javanus* whereas statistically significant reduction in the fecundity of *A. longisetosus* was observed only at 500 ad 1000 times dilution of endosulfan and heptachlor, respectively. Even short duration exposure to pesticide treated soil reduced the fecundity of *C. javanus*. This reproductive sensitivity can be proposed as a potential ecotoxicological tool for pesticide residue detection and soil health assessment in agroecosystems.

Key words : Soil microarthropods – Pesticides – Fecundity – Microcosm – Bioassay.

Introduction

The functional significance of soil biota is hampered due to various anthropogenic factors and pesticide pollution in agricultural soil can be cited as an example. The persistence and bioactivity of pesticide residues on nontarget organisms depend on the type of soil, organic matter content, type of crops, climate and formulation of the chemicals (Martikainen, 1996). In the soil subsystem risk assessment of pesticides is usually made on the basis of responses by decomposer organisms. According to Rawson (1993) bioassay using sensitive soil arthropod species has a well established position in environmental monitoring and impact assessment of toxic chemicals. Ecotoxicological test systems in soil have recently gained importance and an array of procedures are available (Van Straalen and Van Rijn, 1998). However, there is a need for a range of cheaper, easy to use ecotoxicological test

systems that allow both laboratory and field testing. Van Straalen *et al.*, (1995) suggested "Ecotoxicological recovery" as the disappearance of the chemical to a level where it does not have any adverse effects on the soil community. We present a microcosm technique employing the fecundity of soil microarthropod fauna as a suitable bioassay tool in agricultural soil.

Materials and Methods

Commercial formulation of two broad spectrum cyclodiene pesticides namely heptachlor 20EC (Pesticides India) and endosulfan 35EC (Excel Industries Limited) were used for the present laboratory experiments. 4 cosmopolitan microarthropod species namely *Cyphoderus javanus* Borner (Collembola : Insecta), *Archegozetes longisetosus* Aoki, *Lancetoppia confusaria* sp. nov. and *Scheloribates albitalatus* Berlese (all Cryptostigmata : Acari) were

Table 1 : Direct toxicity (mortality 24 hrs.) of sub-agricultural dilutions of pesticides on different soil microarthropod species (Percentage mortality, mean \pm S.D.).

Pesticide	Species	Dilution factor						
		1*	10	100	300	1000	2000	10000
Heptachlor 20 EC	<i>C. javanus</i>	100	100	74.5 \pm 12.9	28.2 \pm 7.5	nil	nil	nil
	<i>L. confusaria</i>	100	17.5 \pm 8.9	nil	nil	nil	nil	nil
	<i>A. longisetosus</i>	100	100	73.3 \pm 12.1	37.8 \pm 13.9	nil	nil	nil
	<i>S. albialatus</i>	100	26.0 \pm 15.2	nil	nil	nil	nil	nil
Endosulfan 35 EC	<i>C. javanus</i>	100	97.6 \pm 4.18	81.6 \pm 13.7	71.3 \pm 11.3	58.9 \pm 11.0	55.0 \pm 7.6	38.3 \pm 3.7
	<i>L. confusaria</i>	100	67.1 \pm 8.8	4.0 \pm 8.0	nil	nil	nil	nil
	<i>A. longisetosus</i>	100	25.7 \pm 5.4	nil	nil	nil	nil	nil
	<i>S. albialatus</i>	82.0 \pm 14.8	16.0 \pm 15.2	nil	nil	nil	nil	nil

*Highest recommended agricultural dose – Heptachlor 20 EC = 16.25 lit/ha; Endosulfan 35 EC = 2.5 lit/ha.

Table 2 : Fecundity of soil microarthropod species under sublethal toxicity of pesticides.

Specimens	Experimental sets	Dose (ml/ha)/dilution	Fecundity				
			3	6	9	12	15
<i>C. javanus</i>	C ₁	—	2.0	6.88	11.69	17.98	26.95
	H ₁	16.25 (1,000)	nil	2.74	3.65	3.65	3.65
	H ₂	8.125 (2,000)	nil	1.78	1.78	3.93	3.93
	C ₂	—	2.0	6.88	11.69	17.98	26.95
	E ₁	0.227 (11,000)	0.92	3.76	7.31	11.11	16.8
	E ₂	0.208 (12,000)	0.80	3.81	8.3	11.52	16.8
<i>A. longisetosus</i>	C ₁	—	1.3	2.76	5.42	12.46	23.93
	H ₁	16.25 (1,000)	0.44	1.08	2.09	5.17	9.28
	H ₂	8.125 (2,000)	0.74	1.93	4.07	9.90	21.30
	C ₂	—	1.3	2.76	5.42	12.46	23.93
	E ₁	12.5 (200)	0.56	2.63	4.03	4.47	4.64
	E ₂	5.0 (500)	0.94	2.69	4.21	5.05	5.63
<i>L. confusaria</i>	C ₁	—	nil	0.08	1.72	2.88	3.72
	H ₁	162.5 (100)	nil	nil	nil	nil	nil
	H ₂	32.5 (500)	nil	nil	nil	0.1	0.95
	C ₂	—	nil	nil	0.20	1.60	5.54
	E ₁	5.0 (500)	nil	nil	0.02	0.65	3.50
	E ₂	3.57 (700)	nil	nil	0.16	1.92	5.34
<i>S. albialatus</i>	C ₁	—	nil	nil	0.38	1.63	6.44
	H ₁	162.5 (100)	nil	nil	nil	0.12	0.70
	H ₂	32.5 (500)	nil	nil	0.26	0.92	2.32
	C ₂	—	nil	nil	0.14	1.30	5.48
	E ₁	50.0 (50)	nil	nil	nil	0.82	1.94
	E ₂	35.7 (70)	nil	nil	nil	0.88	2.97

C₁ and C₂ = Untreated control sets; H₁ = Heptachlor higher dose treated set; H₂ = Heptachlor lower dose treated set; E₁ = Endosulfan higher dose treated set; E₂ = Endosulfan lower dose treated set.

employed to study short term and long term ill effects of these pesticides. They were extracted live from field samples with the help of modified Tullgren funnels and stock reared on soil medium containing baker's yeast as food. The vessels were maintained under controlled temperature (27 \pm 1°C) and moisture (15% approx.) conditions in a BOD incubator. All the experiments were conducted using sterile and powdered (25 mesh) sandyloam soil of known physicochemical

properties, as the substratum (WHC 36%, pH 6.1 \pm 0.0, organic carbon 0.69 \pm 0.01%, nitrate nitrogen 4.1 \pm 0.65 $\mu\text{g g}^{-1}$, calcium 411 \pm 15.6 $\mu\text{g g}^{-1}$, potassium 315 \pm 8.6 $\mu\text{g g}^{-1}$, phosphorus 4.6 \pm 0.25 $\mu\text{g g}^{-1}$).

The highest recommended agricultural dose of heptachlor 20EC (16.25 lit/ha) and endosulfan 35EC (2.5 lit/ha) were diluted in distilled water according to standard water requirement i.e. @ 1,000 lit/ha for bare field soil. A standardized

Table 3 : Statistical significance of the differences among the fecundity of soil microarthropods subjected to sublethal toxicity of pesticides.

Pesticides	Species	Combination		Deviation $\bar{X}_1 - \bar{X}_2$	df	t	p
		X_1	X_2				
Heptachlor 20 EC	<i>C. javanus</i>	C ₁	H ₁	23.3	7	5.9	<0.001
		C ₁	H ₂	23.02	7	5.9	<0.001
		H ₁	H ₂	0.28	8	0.5	n.s.
	<i>A. longisetosus</i>	C ₁	H ₁	14.65	8	3.6	<0.01
		C ₁	H ₂	2.63	8	0.4	n.s.
		H ₁	H ₂	12.02	8	2.0	n.s.
	<i>L. confusaria</i>	C ₁	H ₂	2.77	8	8.6	<0.001
		S. albialatus	C ₁	5.74	8	2.5	<0.05
		C ₁	H ₂	4.12	8	1.7	n.s.
		H ₁	H ₂	1.62	8	1.2	n.s.
Endosulfan 35 EC	<i>C. javanus</i>	C ₂	E ₁	10.15	7	2.4	<0.05
		C ₂	E ₂	10.15	7	3.2	<0.02
	<i>A. longisetosus</i>	C ₂	E ₁	19.29	7	5.9	<0.001
		C ₂	E ₂	18.3	8	4.8	<0.01
		E ₁	E ₂	0.99	7	1.1	n.s.
	<i>L. confusaria</i>	C ₂	E ₁	2.04	8	1.5	n.s.
		C ₂	E ₂	0.2	8	0.1	n.s.
		E ₁	E ₂	1.84	8	1.7	n.s.
	<i>S. albialatus</i>	C ₂	E ₁	3.54	8	2.7	<0.05
		C ₂	E ₂	2.51	8	1.8	n.s.
		E ₁	E ₂	1.03	8	1.4	n.s.

C_1 and C_2 = Untreated control sets. Pesticide dilutions in H_1 , H_2 , E_1 and E_2 sets are given in Table 2.

volume of 340 μ l of pesticide dilution calculated on the basis of surface area of treatment vessel (6.5 cm diameter) containing 50 g soil (1 cm thick layer) was dispensed with the help of a micropipette. Untreated control vessels received a similar amount of distilled water. The objective of short term screening studies was to select sublethal doses for detailed experiments. Only laboratory reared and freshly emerged adult specimens of same age and size groups were used for the studies.

Direct toxicity (24 hours) was estimated from the mortality of specimens exposed to the agricultural and subagricultural concentrations. All the vessels were carefully examined under stereoscopic dissecting microscope and specimens without any sign of life even when touched with a fine needle were counted as dead. The long term experiments were conducted to study the chronic effect of pesticides on fecundity and longevity. The microcosms resembled the treatment vessels but with a regular supply of food and moisture and the number of surviving adults and eggs deposited were counted at regular 3 days intervals. In the field conditions there is always a chance of migration of partially affected specimens to safer

places. Therefore, a simple experiment was conducted to study the fecundity of partially affected specimens by exposing *C. javanus* to sublethal doses of heptachlor 20 EC and endosulfan 35 EC for two different duration (24 hours and 72 hours). After exposure, the specimens were transferred to untreated vessels and observations were made as stated above. The data were statistically analysed using 't' test.

Results

The short term direct toxicity (24 hours) of heptachlor and endosulfan (Table 1) showed high sensitivity of all the test species. Heptachlor 20EC produced quick knockdown effect on *C. javanus* and *A. longisetosus*. The toxicity declined sharply with respect to decreasing concentration and produced no mortality of *C. javanus* and *A. longisetosus* at 1,000 times dilution of the agricultural dose, whereas *L. confusaria* and *S. albialatus* escaped direct toxicity at 100 times dilution. On the otherhand, endosulfan 35EC produced notable mortality of *C. javanus* even at 10,000 times dilution of the agricultural dose but toxicity of subagricultural doses declined drastically against the 3 acarine species. The

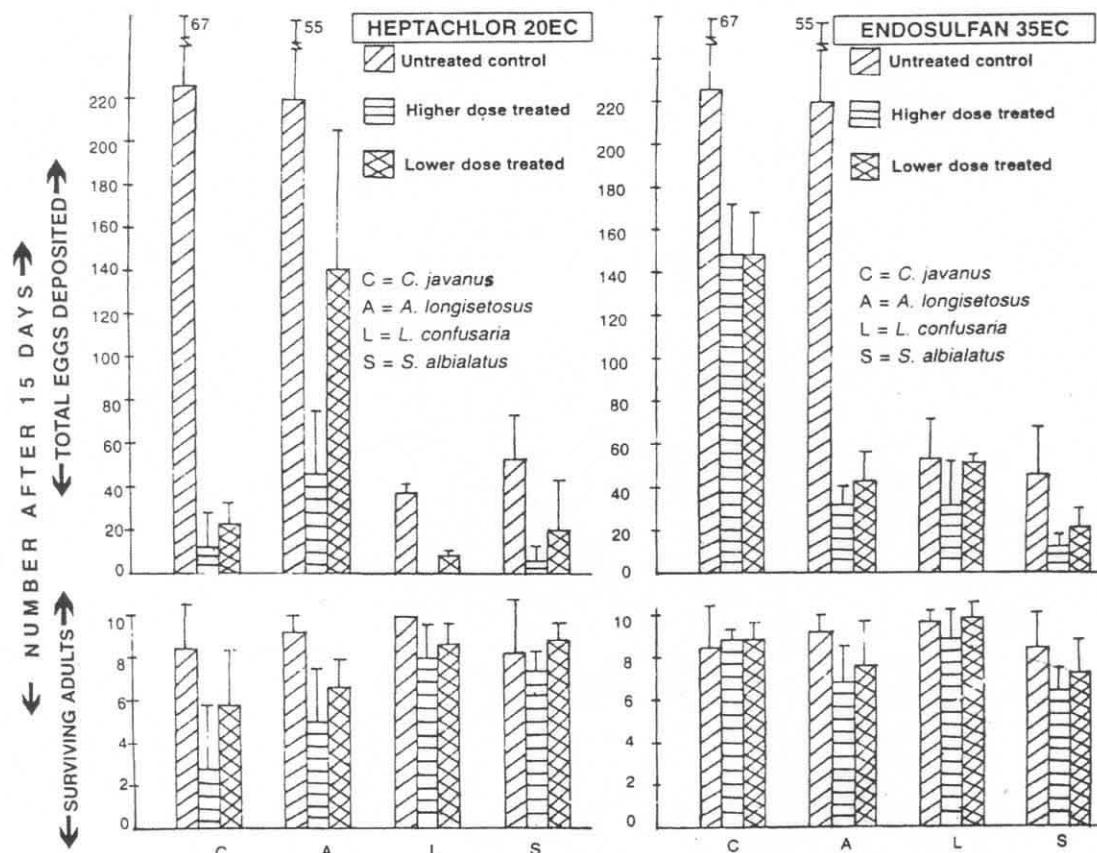


Fig. 1. Comparison of the average number of adults and eggs of soil microarthropod species in different experimental sets.

sensitivity of different test species as evident from the mortality rate was in the order : *C. javanus* > *A. longisetosus* > *L. confusaria* > *S. albitalatus*.

The sublethal toxicity of heptachlor 20EC and endosulfan 35EC on the fecundity of *C. javanus*, *A. longisetosus*, *L. confusaria* and *S. albitalatus* are incorporated in Table 2. In the untreated set *C. javanus* started egg laying within 3 days which steadily increased to reach a high level of 26.95 per surviving adult within 15 days. The chronic toxicity of heptachlor was evident throughout the study and resulted in very low fecundity in both H_1 and H_2 sets having 1,000 and 2,000 times dilution doses of the commercial formulation, respectively. The adverse effect of endosulfan on *C. javanus* with respect to the rate of egg laying also exemplified the high sensitivity from the fact that variation between E_1 and E_2 sets were negligible. The fecundity of *A. longisetosus* went

upto 23.93 in the untreated control set but the rate was severely affected in the H_1 set treated with 1,000 times dilution of heptachlor 20EC. However, the fecundity at 2,000 times dilution was almost similar to that of untreated control. Table 2 also shows comparable and highly remarkable chronic toxicity of 200 and 500 times dilutions of endosulfan 35EC on the fecundity of *A. longisetosus* but the ill effect was evident from the 12th day suggesting somewhat delayed toxicity. The fecundity of *L. confusaria* also demonstrated the sensitivity of reproductive parameter which infact totally arrested egg laying in heptachlor contaminated soil. The chronic toxicity of endosulfan 35EC at 500 times dilution of the agricultural dose decreased the fecundity of *L. confusaria* to notably low level than in the untreated control and E_2 set treated with 700 times dilution. Chronic toxicity of heptachlor 20EC

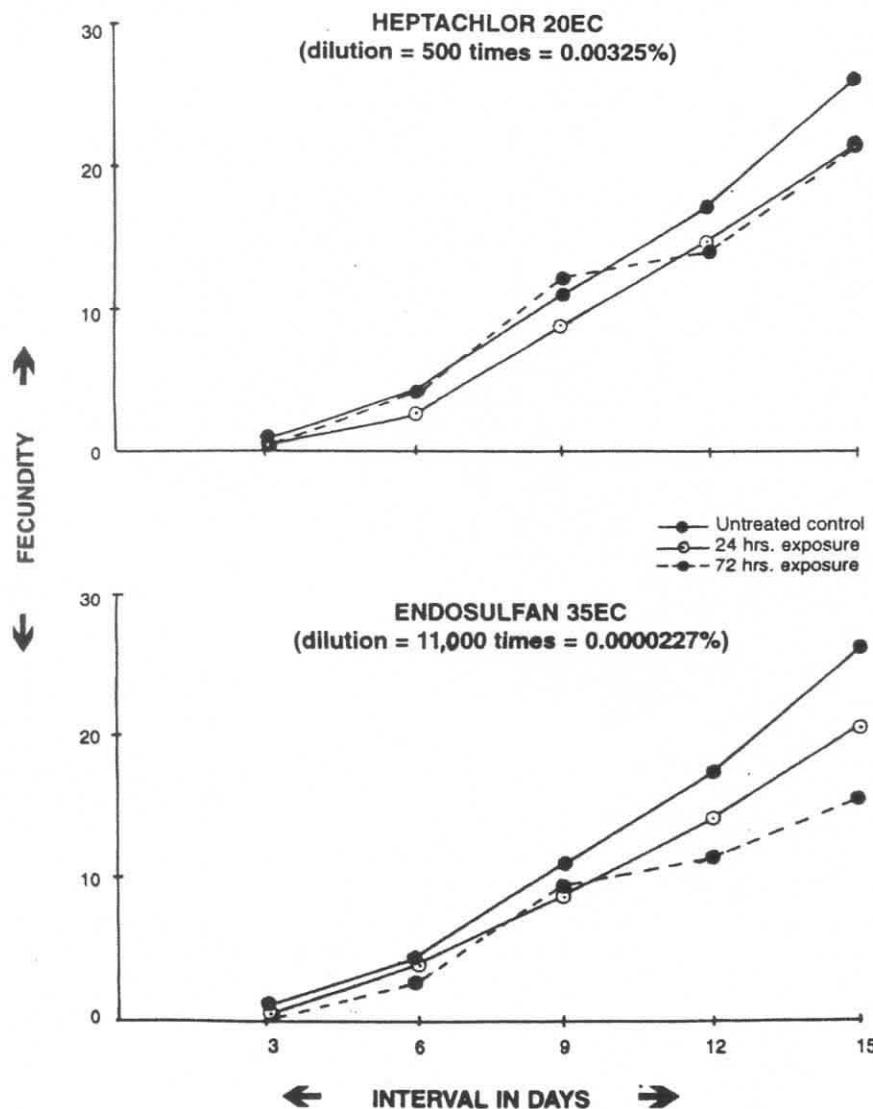


Fig. 2. Effect of short duration exposure to pesticides on the fecundity of *C. javanus*.

and endosulfan 35EC also reduced the fecundity of *S. albitalatus* to very low levels.

An overall comparison between the number of surviving adults and number of eggs deposited in different experimental sets is included in Fig. 1. The chronic toxicity of heptachlor 20EC at 1,000 times dilution of the agricultural dose could kill majority of the adult population but more than 50% adults survived in the set treated with 2,000 times dilution. However, the ill effect on fecundity was evident in both sets. Adult mortality in *A.*

longisetosus was less in both 1,000 and 2,000 times dilution sets of heptachlor 20EC but the rate of egg laying was severely affected at least in the set treated with 1,000 times dilution. Fig. 1 also shows that the adults of *L. confusaria* and *S. albitalatus* could tolerate higher levels of chronic toxicity of heptachlor 20EC but their egg laying was severely affected demonstrating the sensitivity of reproductive parameter. Endosulfan 35EC at 11,000 and 12,000 times dilution of the agricultural dose could not affect the survival rate

of adults of *C. javanus* but the negative effect on its reproductive behaviour was evident from the low number of eggs produced. Similarly, sublethal concentrations at 200 and 500 times dilution produced negligible mortality in the adult population of *A. longisetosus* but the egg laying was reduced to very low levels. *L. confusaria* was the most sensitive acarine species against endosulfan 35EC (Table 2) but Fig. 1 shows that even though 700 times dilution was not harmful as far as the adult survival and fecundity were concerned, 500 times dilution of the agricultural dose could decrease the level of egg production. On the contrary, *S. albitalatus* was the most resistant species to the short term direct toxicity of endosulfan 35EC. The chronic toxicity estimated at 50 and 70 times dilution, however, produced some mortality in the adult population and the rate of egg laying was decreased to significantly low levels.

The statistical significance of the ill effect of heptachlor 20EC and endosulfan 35EC on the overall fecundity of individual species is summarised in Table 3. In the case of heptachlor 20EC, *C. javanus* was more sensitive than *A. longisetosus* and *L. confusaria* was sensitive over *S. albitalatus*. The sensitivity was also evident from the fact that the differences between H_1 and H_2 sets were not statistically significant. *A. longisetosus* was highly sensitive to endosulfan 35EC followed by *C. javanus*, *L. confusaria* and *S. albitalatus*. Here also the differences between E_1 and E_2 sets did not vary indicating high sensitivity of all the species. The study demonstrated that soil microarthropods cannot perform normal rate of reproduction in pesticide contaminated soil. Fig. 2 includes the result of short duration exposure to sublethal concentrations of heptachlor 20EC (500 times dilution) and endosulfan 35EC (11,000 times dilution) on the fecundity of *C. javanus*. Variation in the rate of egg laying between heptachlor 24 and 72 hours exposed sets were negligible, but the effect of endosulfan was more pronounced in 72 hours exposed set. The ill effect of both pesticides was evident from the low rate of egg laying when compared to untreated control sets.

Discussion

Commercial formulation of heptachlor and endosulfan were used for the present experiments because of direct applicability in agroecosystems. Existing literature on ecotoxicological studies

using soil fauna indicate preference to commercial formulation (Van Straalen and Van Gestel, 1993). Similarly, for reasons of ecological realism it is essential that exposure in laboratory tests resembles that in the field and therefore we conducted the present experiments using natural field soil of known physicochemical properties as the medium. According to Loos-Holmin (1982) only representative species of soil fauna living in habitats naturally influenced by pesticides are relevant as test specimens in laboratory experiments when conclusions are to be transferred from the laboratory results to the field situations. As such, laboratory reared populations of nontarget saprotrophic soil microarthropods of same age and size groups were used for the experiments. The suitability of soil arthropod fauna for screening experiments has been reported by several workers and according to Van Straalen and Van Rijn (1998) test protocols for some species of Collembola have already achieved international standardization as bioindicator tools.

Both heptachlor 20EC and endosulfan 35EC could produce mortality of all the test species within 24 hours. According to O'Brien (1967) cyclodiene pesticides are broad spectrum in action and their toxicity does not indicate any marked variation among different invertebrate species in contrast to much variation observed among the compounds for mammalian toxicity. Though Collembola suffered even at very low dilutions, the acaricidal property of both pesticides decreased sharply in subagricultural doses. Van Straalen and Van Rijn (1998) noted that although the effects of chlorinated hydrocarbon pesticides like lindane on aquatic animals and higher terrestrial organisms are well documented, toxicity to arthropods and other soil dwelling species is not completely known. Endosulfan is an insecticide cum acaricide with nonsystemic broad spectrum toxicity whereas heptachlor is used against soil borne pests, irrespective of the fact that it is banned from use in croplands. Tomlin (1975) while comparing the toxicity of several insecticides on Collembola showed that bioactivity of pesticides can vary considerably depending upon the species and the *Onychiurus justiporteri* is more resistant than *Folsomia candida*. Joy and Chakravorty (1991) noticed high sensitivity of *Cyphoderus* sp. to eight different insecticides in the laboratory but another Collembola namely *Xenylla* sp. could escape the ill effect of some insecticides.

Research in Ecotoxicology is principally concerned with toxic effects produced not by the absorption of fairly strong doses over a short period but with exposure to very low concentration of pollutant whose repeated cumulative effects result in far more dangerous perturbation (Ramade, 1987). Van Straalen and Van Rijn (1998) noted that reproduction and growth rate are more relevant end points than mortality because effects on these parameters usually appear at lower exposure concentration. In the present study the chronic sublethal toxicity of both pesticides in suppressing the fecundity of sensitive microarthropods was evident. Among different test species, *C. javanus* demonstrated highest sensitivity against heptachlor and endosulfan and among the 3 acarine test species *A. longisetosus* was the obvious choice. Jepson (1993) while describing a model laboratory based registration testing, recommended for the estimation of the rate of egg production as a valid parameter of toxicity. Krogh (1995) showed that *Folsomia candida* suffered sublethal toxic effect of dimethoate on the rate of reproduction. Sanocka-Woloszyn and Woloszyn (1970) demonstrated that herbicides namely prometryne, aphalon and chlorpropham could distinctly lower the rate of reproduction of *Folsomia candida* within 3 weeks of exposure.

Saprotrophic soil arthropods are easily affected by the toxicant because of direct chance of contamination. In the present microcosm study chronic exposure to sublethal concentration could affect the survival success of adult population depending upon the sensitivity of species and dilution of pesticide. Soil arthropods are able to escape from toxic sites by migration and avoidance. Sorensen *et al.* (1995) and Bayley (1995) observed that exposure to sublethal concentrations of dimethoate applied in natural soil medium could produce hyperactivity on the locomotor behaviour of *Folsomia candida* and *Porcellio scaber*, respectively. Hopkin (1997) while discussing the significance of ecotoxicity assay noted that *Collembola* can always migrate away from an unpleasant environment. The survival and reproduction of such partially affected animals have ecological implications. The present results showed notable decrease in the fecundity of such partially exposed individuals of *C. javanus*, particularly in the case of endosulfan 35EC. Chakravorty and Joy (1996) while comparing the ecological hazard of insecticides on soil *Collembola*

noticed low survival success of partially affected animals in endosulfan treated soil than in methyl parathion treated soil.

The results of present microcosm experiments indicated that agricultural formulations of heptachlor and endosulfan are extremely toxic to nontarget soil microarthropod fauna. Sublethal toxicity of both pesticides could significantly reduce the fecundity of all the 4 species investigated. This reproductive sensitivity can be proposed as a potential ecotoxicological tool for pesticide residue detection and soil health assessment in agroecosystems.

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